Persistence of Meningococcal Antibodies and Response to a Third Dose After a Two-dose Vaccination Series with Investigational MenABCWY Vaccine Formulations in Adolescents

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Background: In a primary study, healthy adolescents received 2 doses (months 0/2) of 1 of the 4 investigational meningococcal ABCWY vaccine formulations, containing components of licensed quadrivalent glycoconjugate vaccine MenACWY-CRM, combined with different amounts of recombinant proteins (rMenB) and outer membrane vesicles (OMV) from a licensed serogroup B vaccine, or 2 doses of rMenB alone or 1 dose of MenACWY-CRM then a placebo.

Methods: This phase 2 extension study evaluated antibody persistence up to 10 months after the 2-dose series and the immunogenicity and safety of a third dose (month 6). Immune responses against serogroups ACWY and serogroup B test strains were measured by serum bactericidal assay with human complement.

Results: At month 12, antibody persistence against serogroups ACWY in all 2-dose MenABCWY groups was at least comparable with the 1-dose MenACWY-CRM group. Bacterial antibodies against most serogroup B test strains declined by month 6, then plateaued over the subsequent 6 months, with overall higher antibody persistence associated with OMV-containing formulations. A third MenABCWY vaccine dose induced robust immune responses against vaccine antigens, although antibody levels 6 months later were comparable with those observed 5 months after the 2-dose series. All investigational MenABCWY vaccines were well tolerated.

Conclusions: Two or three doses of investigational MenABCWY vaccines elicited immune responses against serogroups ACWY that were at least comparable with those after 1 dose of MenACWY-CRM. After either vaccination series, investigational MenABCWY vaccine formulations containing OMV had the highest immunogenicity against most serogroup B test strains. No safety concerns were identified in this study.

Key Words: meningococcal disease, conjugate vaccine, immunogenicity, persistence, safety, adolescents

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Peter Dull is currently at the Bill and Melinda Gates Foundation, Seattle, WA. The trial registration number was ClinicalTrials.gov NCT01367158.

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Infections with pathogenic strains of Neisseria meningitidis, a leading cause of bacterial meningitis and septicemia worldwide, are characterized by a rapid onset and devastating outcomes. Despite the availability of effective antibiotics, case fatality rates remain at approximately 10%, and up to 20% of survivors suffer permanent debilitating sequelae.1 Invasive meningococcal disease (IMD) can affect people at any age, but the highest incidence of disease is among infants, followed by adolescents and young adults, with overall higher case fatality rates among the latter age groups.2 Most cases of IMD are caused by 1 of the 5 immunologically distinct N. meningitidis serogroups (A, B, C, W and Y).3 The epidemiology of meningococcal disease and distribution of these serogroups vary geographically, as well as temporally.4 In the African meningitis belt, for instance, serogroup W epidemics currently account for most cases of IMD,5 whereas in European countries and Australia, sporadic serogroup B-attributable disease accounts for most cases.6 In the US and Latin America, most meningococcal disease occurs sporadically and is attributable to serogroups B, C and Y.

To reduce the burden of IMD, many countries have incorporated meningococcal capsular polysaccharide–protein conjugate vaccines, including monovalent vaccines against serogroups A or C, as well as the more recently developed quadrivalent glycoconjugate vaccines against serogroups ACWY, into mass vaccination campaigns or routine immunization schedules. One available quadrivalent glycoconjugate vaccine is MenACWY–CRM (Menvoe, Novartis Vaccines, Siena, Italy), which contains capsular oligosaccharides from serogroups ACWY conjugated to a nontoxic mutant diphtheria toxoid, CRM197, as the carrier protein. Clinical trials have shown that MenACWY–CRM is highly immunogenic against serogroups ACWY and well tolerated in all age groups, including infants.6,7 MenACWY-CRM is licensed for use in more than 60 countries, including the US and countries in the EU, Latin America and Asia.

Unlike the capsules of serogroups ACWY, the serogroup B capsule is poorly immunogenic.11,12 As an alternative to the glycoconjugate approach, a multicomponent serogroup B vaccine was developed, 4CMenB (Bexsero, Novartis Vaccines, Siena, Italy), containing recombinant forms of subcapsular protein antigens, as well as outer membrane vesicles (OMV) from a New Zealand serogroup B outbreak strain. Analyses of 4CMenB coverage indicate that 4CMenB is immunogenic against the majority of circulating serogroup B strains worldwide,13 and the results of phase 2/3 clinical trials show that it is well tolerated in infants, children, adolescents and adults.14–18 4CMenB is licensed in the EU, Australia, Canada, Chile and Uruguay. In addition, 4CMenB was used under the authorization of an Investigational New Drug application at 2 US universities in 2014 in response to local outbreaks of serogroup B disease,19,20 and was recently approved in the US for use in individuals 10–25 years of age. A combination meningococcal ABCWY vaccine would simplify immunization schedules and could increase
vaccine compliance, as well as improve coverage against the main disease-causing meningococcal serogroups.

An investigational meningococcal ABCWY vaccine, containing glycoconjugate components from MenACWY-CRM combined with different amounts of serogroup B components from 4CMenB, is currently under development. In a previous study (Clinical trials.gov identifier: NCT01210885), the immunogenicity, safety and tolerability of 2 doses of 1 of the 4 candidate MenABCWY vaccine formulations were evaluated in healthy adolescents, in comparison with 2 doses of an investigational serogroup B vaccine formulation or 1 dose of licensed MenACWY-CRM followed by a placebo. In this phase 2 extension study, we assessed the persistence of bactericidal antibodies against meningococcal serogroups ACWY and serogroup B test strains up to 10 months after completion of the 2-dose series. In addition, we assessed the immunogenicity, reactogenicity and safety of a third dose of the same investigational MenABCWY vaccine formulation received in the primary study.

MATERIALS AND METHODS

Study Design

This phase 2 observer-blind, controlled, randomized, extension study was conducted at 6 centers in Panama, 3 in Colombia and 2 in Chile, between July 2011 and July 2012. The protocol was approved by the Ethics Review Committee of participating centers, and written informed consent was obtained from each eligible subject or their parent/legal guardians, as appropriate. The study was undertaken in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice.

Enrolled subjects from the primary study groups I–V (see Fig. 1) were randomized in a 1:2 ratio using a validated, computer-generated random allocation list provided by the sponsor to receive either a third dose of the same meningococcal vaccine formulation administered in the primary study (groups I-3d, II-3d, III-3d, IV-3d, V-3d) or a dose of a tetanus, diptheria and acellular pertussis vaccine (Tdap; groups I-2d, II-2d, III-2d, IV-2d, V-2d), approximately 180 days after the first vaccination in the primary study (at month 6; first and second doses were administered at months 0 and 2, respectively). Subjects randomized to group VI of the primary study (and received 1 dose of MenACWY-CRM and then a placebo dose at months 0 and 2, respectively) received 1 dose of Tdap at month 6. The dosing regimen of the primary series of the investigational MenACWY vaccines for adolescents is based on experience with the licensed 4CMenB vaccine in adolescents, in which 2 doses of 4CMenB, administered 1–6 months apart, elicited robust and sustained bactericidal antibody responses against diverse circulating serogroup B test strains.

The primary immunogenicity objective of this study was to evaluate the bactericidal antibody response against meningococcal serogroups ACWY and serogroup B test strains 1 month after a third dose of the same meningococcal vaccine formulation administered in the primary study. Secondary immunogenicity objectives included (1) assessment of bactericidal antibody persistence at 4, 5 and 10 months after completion of a 2-dose series of investigational MenACWY vaccine formulations, versus control vaccines, and (2) assessment of bactericidal antibody persistence at 6 months after completion of a 3-dose series of investigational MenABCWY vaccine formulations, versus control vaccines. This report focuses on bactericidal antibody persistence following a 2-dose or 3-dose series of investigational MenABCWY vaccine formulations.

Subjects

Of 485 eligible subjects from the primary study, 440 female and male subjects, 11–18 years of age, were enrolled in this study.

Main exclusion criteria were a history of any meningococcal vaccination (except vaccines administered in the primary study), a history of N. meningitidis infection, close contact with an individual with confirmed N. meningitidis infection within 60 days before enrollment, pregnancy or breast feeding, any impairment of immune function, a severe allergic reaction after previous vaccinations, receipt of blood products or an immunoglobulin preparation 90 days before enrollment, receipt of other vaccines up to 4 weeks before enrollment or a plan to receive a vaccine within 4 weeks after administration of the study vaccines. Females of childbearing age were required to commit to the use of appropriate birth control measures for the duration of the study and to confirm use of birth control measures for at least 2 months before study entry.

Vaccines

The investigational MenABCWY formulations were prepared immediately before vaccination by mixing the lyophilized CRM197-conjugated oligosaccharides of serogroups ACWY with 1 or 2 doses of a liquid suspension containing meningococcal serogroup B recombinant proteins Hbpa, NadA and NHBA,21 adsorbed on aluminum hydroxide, (rMenB) and, for those formulations containing the OMV component, a “full dose” or “one-quarter dose” of OMV from serogroup B strain NZ98/254. After reconstitution, all MenABCWY formulations contained 10 µg of MenA-CRM197 and 5 µg each of MenC-CRM197, MenW-CRM197, and MenY-CRM197. The content of the serogroup B components and the total injected volumes were as follows (vaccine description in parentheses):

- Group I-3d [MenACWY-CRM + rMenB (1×)]: 50 µg of each rMenB protein, 1.5 mg of aluminum hydroxide; 0.5 mL dose
- Group II-3d [MenACWY-CRM + rMenB (2×)]: 100 µg of each rMenB protein, 3.0 mg of aluminum hydroxide; 1.0 mL dose
- Group III-3d [MenACWY-CRM + rMenB (1×) + OMV]: 50 µg of each rMenB protein, 1.5 mg of aluminum hydroxide, 25 µg (full dose) of OMV; 0.5 mL dose
- Group IV-3d [MenACWY-CRM + rMenB (1×) + ¼OMV]: 50 µg of each rMenB protein, 1.5 mg of aluminum hydroxide, 6.25 µg (one-quarter dose) of OMV; 0.5 mL dose

All investigational MenABCWY formulations contained the full composition of the MenACWY-CRM parent vaccine (ie, CRM197-conjugated oligosaccharides of serogroups ACWY) combined with different amounts of the serogroup B components from the licensed 4CMenB vaccine. Only the MenABCWY formulation administered to subjects in group III-3d contained the amount of recombinant proteins and OMV equivalent to 4CMenB (ie, 1 dose of rMenB combined with a full dose of OMV).

Group V-3d received 1 dose of the investigational serogroup B formulation, rMenB, which contained 50 µg of each rMenB protein and 1.5 mg of aluminum hydroxide and was administered as a 0.5 mL dose.

The Tdap vaccine (Adacel, Sanofi-Pasteur Limited, Canada) was administered as a 0.5-mL dose according to the package insert. Vaccines were prepared and administered by unblinded personnel who did not participate in any evaluation of subjects. All vaccines were administered by intramuscular injection into the deltoid muscle of the nondominant arm.

Assessment of Immunogenicity

Blood samples (20mL) were obtained at months 6, 7 and 12 (Fig. 1). Levels of bactericidal antibodies were measured by an automated, high throughput serum bactericidal assay with human complement (hSBA) essentially as described previously22 at Novartis Clinical Laboratory Sciences, Marburg, Germany.
Serum bactericidal activity was measured against *N. meningitidis* serogroup ACWY reference strains and against serogroup B test strains chosen to measure immune responses to specific vaccine antigens: 44/76-SL (fHbp), 5/99 (NadA), M07-0241084 (NHBA) and NZ98/254 (PorA P1.4, a major *N. meningitidis* OMV protein).

Although an hSBA titer ≥4 has been shown to be associated with protection against IMD caused by serogroups A and C,23 more conservative titer levels based on results of assay validation were used in this study to avoid overestimation of protection. Specifically, at all timepoints, immune responses were expressed as the percent- age of subjects with hSBA titers ≥8 (serogroups ACWY) or hSBA titers ≥5 (serogroup B test strains) and by hSBA geometric mean titers (GMTs).

**Safety Assessment**

Subjects were monitored for 30 minutes postvaccination for any reactions. Solicited local and systemic reactions, as well as all unsolicited adverse events (AEs), were collected for 7 days after each vaccination. Medically attended AEs, serious AEs (SAEs) and AEs leading to withdrawal from the study were collected during the entire study period (181 days). Investigators assessed AEs for a causal relationship to study vaccination.

**Statistical Methods**

There were no statistical hypotheses associated with the study objectives; all analyses were descriptive. The immunogenicity analyses were performed on the modified intention-to-treat
TABLE 1. Study Population Demographics

<table>
<thead>
<tr>
<th>Group</th>
<th>Population</th>
<th>n</th>
<th>Gender, n (%)</th>
<th>Age, mean ± SD (yr)</th>
<th>Weight, mean ± SD (kg)</th>
<th>Height, mean ± SD (cm)</th>
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</thead>
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<tr>
<td>I-2d</td>
<td>25</td>
<td></td>
<td></td>
<td>14.2 ± 2.0</td>
<td>54.3 ± 11.5</td>
<td>159.7 ± 7.9</td>
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<tr>
<td>II-2d</td>
<td>49</td>
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<td>14.4 ± 2.1</td>
<td>54.3 ± 11.5</td>
<td>159.7 ± 7.9</td>
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<td>III-2d</td>
<td>24</td>
<td></td>
<td></td>
<td>14.2 ± 2.1</td>
<td>54.3 ± 11.5</td>
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<td>54.3 ± 11.5</td>
<td>159.7 ± 7.9</td>
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<tr>
<td>VI</td>
<td>23</td>
<td></td>
<td></td>
<td>14.5 ± 2.0</td>
<td>54.3 ± 11.5</td>
<td>159.7 ± 7.9</td>
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Results

Among 440 enrolled subjects, 428 (97%) completed the study (Fig. 1). Twelve subjects (3%) were withdrawn prematurely from the study because of withdrawal of consent (n = 6), loss to follow-up (n = 5) and protocol deviations (n = 1; administration of a study vaccine during pregnancy).

The baseline characteristics and demographics of the enrolled subjects were comparable across groups (Table 1). The mean age of subjects at enrollment was 14.5 years.

Immunogenicity

Antibody Persistence Against Serogroups ACWY After a 2-dose Vaccination Series

At 1 month after the second vaccination (month 3), the percentage of subjects with hSBA titers ≥5 against each of serogroups ACWY across the 2-dose MenABCWY groups (I-2d, II-2d, III-2d, IV-2d) were comparable to or higher than the respective proportions in the 1-dose MenACWY-CRM group (VI; Fig. 2). At month 12, the percentage of subjects with hSBA ≥8 against serogroups CWY in groups I-2d, II-2d, III-2d and IV-2d remained high (78–98%) and were overall higher than group VI (60–90%); whereas the proportions against serogroup A were comparable across groups (46–63%). GMTs against each serogroup at each of months 3, 6, 7 and 12 for groups I-2d, II-2d, III-2d and IV-2d were comparable across groups (46–63%). GMTs against each serogroup at each of months 3, 6, 7 and 12 for groups I-2d, II-2d, III-2d and IV-2d were comparable with or higher than those of group VI (Fig. 3).

Two doses of rMenB initially elicited substantial immune responses against serogroups ACW at month 3, but GMTs were lower than those of the MenABCWY vaccines and decreased to baseline levels by month 12 (group V-2d; Figs. 2 and 3).

Antibody Persistence Against Serogroup B Test Strains After a 2-dose Vaccination Series

At 1 month after the second vaccination (month 3), the percentage of subjects with hSBA titers ≥5 against the fHbp test strain was high across the 2-dose MenABCWY groups and the 2-dose rMenB group (90–100%). These percentages rapidly declined by month 6 (48–58%) and then decreased less substantially over the subsequent 6 months (Fig. 4). A similar pattern was observed for the NHBA test strain, with the highest proportion of subjects with hSBA titers ≥5 at month 3 in groups III-2d, IV-2d and V-2d (61–73%), when compared with groups I-2d and II-2d (40–50%). Across groups I-2d, II-2d, III-2d, IV-2d and V-2d, the percentage of subjects with hSBA titers ≥5 against the NHBA test strain declined by month 6 (30–46%) and then decreased less substantially over the subsequent 6 months (Fig. 4). For the PorA test strain, initial immune responses at month 3 were only evident in the groups that received OMV-containing formulations (III-2d and IV-2d: 70–79%). Percentages in groups III-2d and IV-2d declined by month 6 (19–23%); however, there was no substantial difference in the percentages for these groups between months 6 and 12. For the NadA test strain, 85–100% of subjects in groups I-2d, II-2d, III-2d, IV-2d and V-2d maintained hSBA titers ≥5 through month 12.

GMTs against each of the serogroup B test strains correlated with the proportions of subjects with hSBA titers ≥5 against the respective strains (Fig. 5). At 5 months after the second dose (month 7), GMTs against the fHbp test strain were highest in group IV-2d, with ranges of 3.6 to 11 across the 2-dose MenABCWY groups and 2-dose rMenB.
group; by month 12, GMTs against the fHbp strain declined and ranged from 2.3 to 4.3 across these groups. For the NadA test strain, the highest GMTs at month 7 were observed in group II-2d, with ranges of 44–96 across the 2-dose MenABCWY groups and 2-dose rMenB group; by month 12 GMTs against the NadA strain ranged from 30 to 46 across these groups. The highest GMTs against the NHBA and PorA test strains at 5 months after the 2-dose series (month 7) were observed in groups III-2d and IV-2d (NHBA: 5.2–6.6; PorA: 2.5–2.6), with no substantial difference in GMTs against these strains from months 7 to 12.

**Immune Responses to a Third Dose and Antibody Persistence Following a 3-dose Vaccination Series, Serogroups ACWY**

At month 6, immediately before the third dose a high proportion of subjects across MenABCWY groups I-3d, II-3d, III-3d and IV-3d had hSBA titers ≥8 against serogroups CWY (92–100%), whereas proportions against serogroup A were lower (52–79%; Fig. 6). One month after the third vaccination (month 7), nearly all subjects in...
The 3-dose MenABCWY groups had hSBA ≥8 against each of the 4 serogroups. At 6 months after the third dose (month 12), all subjects in the 3-dose MenABCWY groups had hSBA titers ≥8 against serogroups CWY and 76–86% maintained hSBA ≥8 against serogroup A.

A third dose of the MenABCWY vaccine formulations increased hSBA GMTs against each of the 4 serogroups at month 7, with increases relative to month 6 of 8- to 15-fold (A), 3- to 5-fold (C), 3- to 4-fold (W) and 3- to 4-fold (Y). GMTs declined by month 12, but remained well above the level associated with seroprotection (≥8) and were higher than the respective GMTs of the 1-dose MenACWY-CRM group (Fig. 7).

One month after a third dose of rMenB (month 7), 73–91% of subjects in group V-3d achieved hSBA ≥8 against serogroups ACW, compared with 33–55% at month 6 (Fig. 6). By month 12, these percentages declined to 45–65%. Immune responses against the serogroup Y reference strain were low (14% at month 7). Similar patterns were seen when immune responses were measured by GMTs (Fig. 7).
Immune Responses to a Third Dose and Antibody Persistence After a 3-dose Vaccination Series, Serogroup B Test Strains

At 1 month after a third dose of a MenABCWY vaccine formulation or the investigational rMenB formulation (month 7), a robust response against the fHbp, NHBA and PorA serogroup B test strains was evident, although the percentages of subjects with hSBA titers ≥5 against the NHBA and PorA strains were substantially higher in groups that received an OMV-containing formulation (III-3d and IV-3d), when compared with groups that received formulations without OMV (I-3d, II-3d, V-3d; Fig. 8). By month 12, the proportion of subjects with hSBA titers ≥5 against the fHbp strain declined relative to month 7, with the highest proportion of subjects being in group III-3d (71%), followed by groups II-3d and V-3d (both 52%). Percentages of subjects with hSBA titers ≥5 against the NHBA and PorA strains also declined by month 12, with the highest proportions in groups III-3d and IV-3d (NHBA: 57–60%; PorA: 20–43%). Across all 3-dose MenABCWY groups,
as well as the 3-dose rMenB group, the percentage of subjects with hSBA titers ≥5 against the NadA test strain were high before the third dose and remained high at month 12 (96–100%).

The hSBA GMTs against serogroup B test strains correlated with the percentage of subjects with hSBA titers ≥5 against the respective serogroup B test strains (Fig. 9). At 6 months after the third dose (month 12), the highest GMTs against the fHbp, NHBA and PorA test strains were observed in group III-3d; GMT ranges across the 3-dose MenABCWY groups and 3-dose rMenB group were 5.4–22 (fHbp), 2.8–10 (NHBA) and 1.4–5 (PorA). The highest GMTs against the NadA test strain at 6 months after the third dose (month 12) were observed in groups II-3d and V-3d, with ranges of 126–274 across the 3-dose MenABCWY groups and 3-dose rMenB group.

**Safety**

Among 440 enrolled subjects, all received at least 1 study vaccination and were included in the safety analysis, and 439 contributed to the analyses of solicited local and systemic reactions occurring within 7 days of vaccination (Table 2). One subject was excluded from the safety analyses because of pregnancy.
Across all study groups, 71–92% of subjects reported at least 1 solicited reaction, with an overall higher frequency of any reaction in groups receiving an OMV-containing MenABCWY formulation (III-3d and IV-3d: 92% for both groups vs. other study groups: 71–83%; Table 3). Solicited local and systemic reactions were reported by 60–92% and 42–80%, respectively, of subjects across study groups, with the highest frequencies in groups III-3d and IV-3d.

Injection site pain was the most common local reaction, with the highest frequencies in groups administered OMV-containing formulations (III-3d: 92% including 36% severe; IV-3d: 79% including 13% severe; Table 2), when compared with those groups administered MenABCWY formulations without OMV or the group administered rMenB (I-3d, II-3d, V-3d: 63–75% including 8–13% severe) or Tdap (60–73% including 6–14% severe).

The most common systemic reactions across groups were myalgia (38–60%), headache (21–42%) and malaise (16–32%; Table 2). Frequencies of individual systemic reactions were comparable across all groups, with the exception of higher frequencies of...
arthralgia and myalgia in groups III-3d and IV-3d, when compared with other study groups (arthralgia: 24–33% vs. 6–21%, respectively; myalgia: 58–60% vs. 25–48%, respectively). Fever (≥38°C) was reported by up to 13% of subjects across groups, with the highest rates in groups I-3d, III-3d and IV-3d. The frequencies of subjects who received analgesics/antipyretics within 7 days postvaccination were higher in groups receiving OMV-containing formulations (III-3d and IV-3d: 20–21%) than among groups receiving MenABCWY formulations without OMV or the group administered rMenB (I-3d, II-3d, V-3d: 8–17%) and the groups that received Tdap (6–12%).

During the entire study period, 107 (24%) of subjects reported at least 1 unsolicited AE, with comparable frequencies of AEs between groups. The most commonly reported AEs were nasopharyngitis (5%) and headache (2%).

A total of 5 SAEs were reported by 5 subjects during the study, with all but 1 assessed as unrelated to the study vaccination. In 1 case,
a 13-year-old girl in group IV-3d (MenACWY-CRM + rMenB + ⅔OMV formulation), experienced severe postvaccination pain, myalgia, headache, chills and fever within 3 hours of vaccination. These events were reported as a SAE that resolved by the following day after outpatient administration of analgesics. There were no reported AEs leading to premature withdrawal from the study, but there was 1 withdrawal because of pregnancy in group I-2d; the pregnancy was identified after the subject received 1 dose of Tdap, and the pregnancy outcome was normal. No deaths were reported in this study.

**DISCUSSION**

Because of the rapid onset and progression of meningococcal disease, persistence of circulating bactericidal antibodies after meningococcal vaccination is important for vaccine effectiveness.24 Here, we report the results of the first study to assess the persistence of immune responses against serogroups ACWY and serogroup B test strains for fHbp (44/76-SL), NadA (5/99), NHBA (M07-0241084) and PorA (NZ98/254) at months 0, 1, 3, 6, 7 and 12, by group. Subjects in groups I-3d, II-3d, III-3d, IV-3d and V-3d were administered 3 doses of the indicated vaccine formulation at months 0, 2 and 6. Subjects in group VI were administered 1 dose of MenACWY-CRM at month 0, a placebo dose at month 2 and 1 dose of Tdap at month 6.
Compared with previous studies, which have demonstrated persistence against serogroup B test strains for 2 months after the second vaccination, this study found a longer duration of protection against non-B serogroups, with titers remaining above the target level for at least 12 months after the second vaccination.

Comparisons of immune response and antibody persistence after 2 doses of MenABCWY versus 2 doses of MenACWY-CRM are needed to determine which of these mechanisms is responsible for the observed difference in antibody persistence.

Across the 2-dose MenABCWY groups, titers of bactericidal antibodies against most serogroup B test strains declined between 1 and 4 months after the second vaccination (months 3–6) and then decreased less substantially over the next 6 months (months 6–12). Although it is certainly possible that these results reflect limited long-term protection of the vaccine, it is important to recognize that there is no established serocorrelate of protection against serogroup (serogroup A) or higher than (serogroups C, W and Y) that of a single dose of the MenACWY-CRM vaccine. The overall greater persistence against non-B serogroups could be attributed to the receipt of 2 doses of MenABCWY formulations, compared with a single dose of MenACWY-CRM, or to induction, by MenABCWY vaccine, of bactericidal antibodies against subcapsular protein antigens, which contribute to killing of these particular test strains. Comparisons of immune response and antibody persistence after 2 doses of MenABCWY versus 2 doses of MenACWY-CRM are needed to determine which of these mechanisms is responsible for the observed difference in antibody persistence.

Across the 2-dose MenABCWY groups, titers of bactericidal antibodies against most serogroup B test strains declined between 1 and 4 months after the second vaccination (months 3–6) and then decreased less substantially over the next 6 months (months 6–12). Although it is certainly possible that these results reflect limited long-term protection of the vaccine, it is important to recognize that there is no established serocorrelate of protection against serogroup

FIGURE 9. hSBA GMTs against serogroups ACWY after a 3-dose series. hSBA GMTs against serogroup B test strains for fHbp (44/76-SL), NadA (5/99), NHBA (M07-0241084) and PorA (NZ98/254) at months 0, 1, 3, 6, 7 and 12, by group. Subjects in groups I-3d, II-3d, III-3d, IV-3d and V-3d were administered 3 doses of the indicated vaccine formulation at months 0, 2 and 6. Subjects in group VI were administered 1 dose of MenACWY-CRM at month 0, a placebo dose at month 2 and 1 dose of Tdap at month 6.
### TABLE 2. Number and Percentage of Subjects With Any or Severe Local or Systemic Solicited Reactions Within 7 Days of the Third Vaccination, by Group (Safety Population for Solicited Reactions)

<table>
<thead>
<tr>
<th>Vaccine Administered at Month 6</th>
<th>Group I-3d (n = 24)</th>
<th>Group I-2d (n = 48)</th>
<th>Group II-3d (n = 24)</th>
<th>Group II-2d (n = 49)</th>
<th>Group III-3d (n = 25)</th>
<th>Group III-2d (n = 48)</th>
<th>Group IV-3d (n = 24)</th>
<th>Group IV-2d (n = 50)</th>
<th>Group V-3d (n = 23)</th>
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<td>4 (8)</td>
<td>1 (4)</td>
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<td>1 (4)</td>
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<td>4 (5)</td>
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<td><strong>Other indicators of reactogenicity n (%)</strong></td>
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<td>Medically attended fever</td>
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<td>0</td>
</tr>
<tr>
<td>Medication used to treat fever</td>
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<td>2 (8)</td>
<td>3 (6)</td>
<td>5 (20)</td>
<td>3 (6)</td>
<td>5 (21)</td>
<td>3 (6)</td>
<td>3 (13)</td>
<td>5 (10)</td>
<td>9 (12)</td>
</tr>
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<td>Medication used to prevent fever</td>
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<td>2 (8)</td>
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<td>0</td>
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<tr>
<td>Stayed home</td>
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<td>3 (13)</td>
<td>1 (2)</td>
<td>0</td>
<td>0</td>
<td>3 (4)</td>
</tr>
</tbody>
</table>

The severity of injection site pain and systemic reactions was described as mild (transient with no limitation in normal daily activities), moderate (some limitation in daily activities) or severe (unable to perform normal daily activities).
TABLE 3. Overview of Solicited Reactions Reported Within 7 Days After Vaccination (Safety Set)

<table>
<thead>
<tr>
<th>Group</th>
<th>I-3d (n = 24)</th>
<th>Group I-2d (n = 48)</th>
<th>Group II-3d (n = 24)</th>
<th>Group II-2d (n = 48)</th>
<th>Group III-3d (n = 24)</th>
<th>Group III-2d (n = 48)</th>
<th>Group IV-3d (n = 24)</th>
<th>Group IV-2d (n = 48)</th>
<th>Group V-3d (n = 50)</th>
<th>Group V-2d (n = 50)</th>
<th>Group VI (n = 438)</th>
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</thead>
<tbody>
<tr>
<td>Any</td>
<td>19 (79)</td>
<td>37 (77)</td>
<td>20 (83)</td>
<td>35 (71)</td>
<td>23 (92)</td>
<td>36 (75)</td>
<td>22 (92)</td>
<td>36 (72)</td>
<td>18 (78)</td>
<td>40 (80)</td>
<td>55 (75)</td>
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<tr>
<td>Local</td>
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<td>33 (69)</td>
<td>18 (75)</td>
<td>35 (71)</td>
<td>23 (92)</td>
<td>36 (75)</td>
<td>20 (83)</td>
<td>30 (60)</td>
<td>17 (74)</td>
<td>38 (76)</td>
<td>53 (73)</td>
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<tr>
<td>Systemic</td>
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<td>33 (69)</td>
<td>14 (58)</td>
<td>29 (51)</td>
<td>20 (80)</td>
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<td>18 (75)</td>
<td>24 (48)</td>
<td>14 (61)</td>
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<tr>
<td>Others*</td>
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<td>8 (17)</td>
<td>3 (13)</td>
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<td>7 (29)</td>
<td>4 (8)</td>
<td>3 (13)</td>
<td>5 (10)</td>
<td>11 (15)</td>
</tr>
</tbody>
</table>

*Refers to other indicators of reactogenicity including subjects who stayed at home because of any reaction and use of analgesic/antipyretic medications.

B N. meningitidis strains, and that hSBA is known to underestimate immunity against serogroup B strains.25 Although administration of a third dose of MenABCWY vaccine formulation substantially increased hSBA GMTs and the percentage of subjects with hSBA titers ≥25 against the fHbp, NHBA and PorA test strains 1 month postvaccination, immune responses against these strains declined by 6 months postvaccination. Waning of GMTs against these strains followed a similar pattern to that observed after administration of the 2-dose series, and comparable levels of antibodies against most serogroup B test strains were observed at 5–6 months after completion of either vaccination series. Although this study was not powered to directly compare the immunogenicity of the 2-dose and 3-dose series, these findings indicate that there may not be a significant advantage to administration of a 3-dose primary vaccination series, in terms of antibody persistence. Indeed, analyses of long-term antibody persistence in adolescents following different dosing regimens of 4CMenB demonstrated that 2 doses of 4CMenB, administered 1–6 months apart, provided sustained, high levels of bactericidal antibodies against the evaluated serogroup B test strains.26

Although there were no observed differences between the MenABCWY formulations in terms of immune responses against serogroups ACWY, there were some differences in immunogenicity against the serogroup B test strains. With regards to hSBA GMTs, for all persistence timepoints following either 2 or 3 doses, the highest titers against the fHbp, NHBA and PorA test strains were observed in subjects who received the OMV-containing MenABCWY vaccine formulations. The highest GMTs against the NadA test strain were observed in the group that received the MenABCWY formulation containing 2 doses of rMenB proteins; however, immune responses against this strain were high across all MenABCWY groups. Overall, doubling the dose of rMenB proteins did not appear to offer much added benefit in terms of immunogenicity; a single dose of recombinant proteins is sufficient to achieve maximal immunological response. A further increase in immune response is only afforded by the addition of the OMV, presumably through an adjuvant effect. Persistence data across all serogroup B test strains for both dosing regimens in this study showed a consistent pattern of greater overall immunogenicity with the full OMV MenABCWY formulation over the one-quarter OMV formulation, as well as over the MenABCWY formulations without the OMV components.

In this study, a third dose of rMenB vaccine was found to elicit immune responses against serogroup A, C and W assay strains but not the serogroup Y strain. The susceptibility of any meningococcal strain to the bactericidal activity of antibodies elicited by the recombinant proteins in the rMenB formulation depends on the degree of similarity between each vaccine antigen and the corresponding protein expressed by the target strain, as well as the expression level of the protein.27,28 These factors can be measured using the enzyme-linked immunoabsorbent assay-based meningococcal antibody typing system, which was developed to relate antigen profiles of different meningococcal strains to killing of the strains by hSBA assay.

The test strains used for serogroups A, C and W in this study each express at least 1 protein with similarity to the rMenB antigens, with a meningococcal antigen typing system relative potency level indicative of bactericidal activity.29 Additional analyses would be needed to define rMenB vaccine coverage against circulating non-serogroup B strains and the clinical significance of these antibody responses.

In this study, the frequencies of reactogenicity were higher in groups receiving OMV-containing vaccines, in terms of injection site pain, erythema, myalgia and arthralgia. However, this disadvantage of the OMV-containing formulations is balanced by the benefit of overall higher antibody levels against serogroup B test strains at 10 months after vaccination. Reactogenicity profiles were similar between the full and one-quarter dose OMV formulations and were comparable with those reported previously for the licensed 4CMenB vaccine.22 OMV-containing meningococcal vaccines are associated with higher rates of local pain, myalgia and arthralgia, although they are regarded as being well-tolerated with no identified safety concerns when used across age groups.30,31 In this study, the overall drop-out rate was low (<3% of enrolled subjects), and no safety concerns were identified for any of the investigational MenABCWY vaccines.

This study has a number of limitations that must be acknowledged. In particular, this is the first study to evaluate the persistence of immune responses to MenABCWY vaccine formulations and as such, no formal hypotheses were tested. In addition, immunogenicity results are based on historical thresholds (hSBA titers ≥28 for serogroups ACWY and ≥25 for serogroup B test strains) and not on the strain-specific lower limits of quantitation, which might be a more appropriate measure considering differences between the test strains.22 Moreover, as a serocorrelate of protection has not been established for serogroup B strains, the clinical relevance of the hSBA assay in assessing sustained effectiveness of these formulations must be further evaluated.23,24 For these reasons, the presented data may underestimate bacterial antibody persistence for certain strains. An additional limitation of this study is that the selected serogroup B test strains do not represent all B strains and, as such, caution may be needed in generalization of the study results. Additional analyses using a larger panel of serogroup B test strains will be needed to define vaccine coverage against circulating serogroup B strains. Finally, given the relatively small numbers of subjects within each vaccination group, as dictated by the number of participants in the primary study, the study was not designed to detect statistically significant differences between groups. Comparison of results across groups must be interpreted with caution given the small sample size and the risk of identifying chance findings in the setting of multiple comparisons. Larger studies are needed to substantiate the results of this study. Moreover, as the rMenB vaccine formulation, which does not contain OMV components, was used as a control investigational formulation for serogroup B in this study, additional studies may be needed to compare MenABCWY formulations to the licensed 4CMenB vaccine, in terms of immune response against serogroup B test strains.

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CONCLUSIONS

All investigational MenABCWY vaccine formulations were immunogenic against serogroups ACWY and serogroup B test strains, with overall greater immunogenicity observed for the OMV-containing vaccine formulations. A third dose of MenABCWY vaccine formulation increased immune responses against serogroups ACWY and serogroup B test strains, although waning of bactericidal antibody levels following a 2-dose and 3-dose vaccination series followed a similar pattern and comparable antibody levels were observed at 5–6 months after each vaccination series. All of the investigational MenABCWY vaccine formulations were generally well tolerated with no identified safety concerns.

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Author’s contributions: J.S. and P.M.D. designed the study, X.S.-L., D.C.A.V. and K.A. conducted the study and participated in the acquisition of data. All authors participated in the analysis and interpretation of the data. EM provided biostatistic expertise.

REFERENCES